**PCR Protocol**

**First, ensure that all samples have been processed and prepared via DNA extraction.**

**Supplies required:** Gloves, 10/200/1250uL pipette tips, P10/200/1000 micropipette, PCR Template, DNA samples, PCR reagents, Ice, Cold tray.

1. Make sure you have PCR template sheets printed out for each reaction you are running.
2. Retrieve Ice from the 3rd floor autoclave room.
3. Based on the information on the PCR template sheet, retrieve and organize any DNA samples, reagents, and primers that will be used in the reaction, excluding Taq Polymerase.
4. Allow reagents/samples to thaw.
   1. 10X buffer: thaw and place on ice
   2. Q solution (Optional): place directly on ice from storage
   3. dNTP: thaw **briefly** and place on ice
   4. Primers: thaw and place on ice
   5. gDNA (+Control): thaw and place on ice
   6. PCR H2O (-Control): keep in tray
   7. Taq Polymerase: **DO NOT REMOVE TAQ FROM THE FREEZER YET**
5. Determine the number of 8 strip tubes necessary for the reaction, and retrieve them with matching tops. (Use the black lines that segregate blocks on the PCR template)
6. Label the first and last tube on each strip tube to its corresponding position as well as the date and reaction name. (1/8 9/16 etc.)
7. Retrieve a 1.5 mL tube to use in making a master mix.
8. Create a master mix using any reagents or primers shared between multiple wells.
   1. Vortex reagent/primer
   2. Add reagent/primer to master mix tube
   3. Discard pipette tip
   4. Cross reagent/primer off PCR template
   5. Repeat until complete, **EXCLUDING TAQ POLYMERASE**
9. Check the DNA you’ve retrieved, make sure that it follows the order outlined by the PCR template.
10. Retrieve a cold tray from the freezer and place strip tubes in order in it.
11. Load DNA samples (use 0.9 uL if 0.8 is listed), load so that the small bead of suspended DNA sticks near the top of the strip tube in a droplet. **Make sure to change pipette tips each sample.**
12. Get taq polymerase from the freezer, do not wait for it to thaw as it will already be liquid, vortex it, and add it to the master mix. Then, immediately put taq polymerase back into the freezer.
13. Vortex the master mix, and add enough to each strip tube to make up the full reaction volume (15uL-DNAuL). Add the master mix in a way that it can wash the DNA down the side of the strip tube.
14. Tap the strip tubes down slightly to allow the solutions to mix in the bottom, then cap the strip tubes.
15. Vortex and centrifuge the strip tubes to properly mix the solution.
16. Go to a thermal cycler and place samples balanced in the cycler.
17. Set a thermal cycling protocol based on the information on the PCR template, then press start.
18. Replace all reagents and samples and clean any equipment used.
19. When the PCR run is complete, label a PCR tray and refrigerate the products.